Central Leptin Regulation of Obesity and Fertility

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Abstract The current obesity epidemic and lack of efficient therapeutics demand a clear understanding of the mechanism underlying body weight regulation. The cloning of leptin, a key body weight regulating adipokine released in proportion to the adipose tissue mass, has revolutionized our understanding of the mechanism by which body weight is controlled. It is now well established that the brain, especially the hypothalamus, maintains body weight homeostasis by effectively adjusting food intake and energy expenditure in response to changes in levels of various nutritional status indicators, including leptin. In addition, one major defect in physiology associated with obesity is reduced fertility. Defects in leptin action result in both obesity and infertility, suggesting that leptin may serve as a link between nutrition supply and reproduction. This review reports recent research advance in neural pathways underlying leptin action on body weight and fertility, and discusses the remaining outstanding challenges.

Keywords Leptin · Obesity · Reproduction · Neurotransmitter · Neuropeptide · Hypothalamus · Fertility · Regulation

Introduction

The last decades have witnessed an alarming obesity epidemic and it is projected that, by year 2030, nearly half of Americans will be obese [1]. Obesity induces an array of metabolic syndromes including type II diabetes mellitus, hypertension, stroke, coronary heart disease as well as compromised reproductive ability [2]. As a result, the economic cost imposed by obesity has become a substantial burden to society, which signifies an urgent need for efficient therapeutics to prevent and treat obesity [1, 3]. Body weight homeostasis is achieved by complex interactions between the brain and peripheral tissues, and is maintained by balanced energy intake (food intake) and energy expenditure [4]. Obesity develops when energy intake exceeds energy expenditure. One key component of the interaction is leptin, an adipokine indicating energy storage and secreted in proportion to the adipose tissue mass. Leptin mainly acts on brain neurons to inhibit feeding and increase energy expenditure. Recent research aided by mouse genetics has gained substantial progress in understanding neural pathways mediating leptin action. In addition, emerging studies demonstrate that leptin, a signal for energy reserve, also directly regulates reproduction. As diagrammed in Fig. 1, this review briefs recent advance in brain mechanisms underlying leptin action on body weight homeostasis and reproduction.

Leptin and its Receptors

Prior to the cloning of leptin, the mechanism of body weight regulation was largely unknown. Nonetheless, studies based on crude lesion approaches identified the hypothalamus as a key brain region responsible for body weight homeostasis. Fine lesion studies also established particular subregions within the hypothalamus as either satiety or hunger centers. However, these studies failed to provide an understanding at

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Fig. 1 This diagram shows major leptin action sites in the brain. Neuronal identity of each subsets of leptin-receptor expressing neurons is color coded

the molecular level. In 1994, the cloning of leptin, a 16 KD protein secreted from adipocytes, has revolutionized our understanding of body weight regulation and provided a molecular tool for studies on body weight homeostasis. It is now well established that leptin is a key regulator of body weight. Leptin, secreted in approximate proportion to the adipose tissue mass, serves as an indicator of body energy reserve, which normally is fat tissue. Increased leptin levels (increased body weight) curtail food intake and stimulate energy expenditure. On the contrary, reduced leptin levels (reduced body weight) stimulate food intake and reduce energy expenditure. The importance of leptin is manifested by mice with deficiency in leptin (*ob/ob*) exhibit severe obesity, hyperphagia, reduced energy expenditure, diabetes as well as loss of reproduction [5]. Consistently, a similar array of abnormalities are also observed in subjects with inactivation of leptin [6], demonstrating that mice and humans share a common leptin pathway. Importantly, obesity and reproduction defects by leptin deficiency can be rescued by pharmacological administration of leptin, demonstrating the reversibility of leptin function.

Leptin action is mediated by leptin receptors. Currently, there are six isoforms of leptin receptors, which are produced by alternative splicing from a single gene. The function of leptin is mainly mediated by the B isoform of leptin receptors (denoted as LepRb) since a similar phenotype to leptin deficiency was observed in mice (*db/db*) and humans with LepRb inactivation [7–9]. Despite strong evidence showing LepRb expression in other sites, the brain is the major site that mediates the function of leptin since brain-

specific deletion of LepRb largely recapitulates phenotypes with whole body LepRb deletion [10]. Reciprocally, abnormalities of the *db/db* mouse are markedly improved by transgenic replacement of central LepRb [11]. Consistent with this, liver-specific deletion of LepRb produces little effects [10].

Leptin Receptor-Expressing Neurons and their Functions in Mediating Leptin Action on Body Weight

Within the brain, the hypothalamus exhibits the most abundant expression of LepRb [12]. Outside the hypothalamus, LepRb is only expressed in scattered neurons in the cortex, hippocampus, periaqueductal grey, dorsal raphe, the hindbrain and other sites [13, 14]. Within the hypothalamus, the arcuate nucleus (Arc) shows the highest density of LepRb expression while the ventral premammillary nucleus (PMV), ventromedial hypothalamus (VMH), the dorsal medial hypothalamus (DMH) and preoptic area (POA) also show prominent expression [13, 14]. Consistent with LepRb expression in the brain, nuclear p-STAT3 expression induced by leptin activation is abundantly expressed in the hypothalamus, and to a lesser degree in other brain regions such as hindbrain and midbrain areas. Collectively, the hypothalamus is the major site in the brain that mediates leptin action on energy balance regulation.

Given the highest degree of expression in LepR expression and p-STAT3 expression in response to leptin action in the Arc, and closest vicinity of these neurons to the median

eminence (ME), the Arc LepRb neurons have long been postulated to be the major mediator of leptin action. Indeed, specific re-expression of *LepRb* in the Arc dramatically rescues obesity phenotype of *db/db* mice [15]. The most recognized function of Arc neurons in mediating leptin action is that mediated by proopiomelanocortin (POMC) and agouti-related peptide (AgRP) neurons, two key groups of neurons of the melanocortin system. Indeed, direct leptin action on POMC and AgRP neurons has been revealed by the obesity phenotypes caused by specific deletion of LepRb in POMC and AgRP neurons [16, 17]. Interestingly, simultaneous deletion of LepRb in POMC and VMH or AgRP neurons produces an additive effect on obesity over individual group [17, 18], suggesting that neural pathways involving these neurons in mediating leptin action on body weight are largely independent.

Given the well-established role of POMC and AgRP neurons in feeding and body weight regulation, the effect of LepRb deletion in POMC and/or AgRP neurons on body weight is surprisingly small, relative to that produced by LepRb deficiency [16, 17]. One possibility is that LepRb expression in these neurons is important; but specific deletion of LepRb in these neurons in early embryonic stages induces developmental compensation from redundant pathways. This possibility appears not to be the case for POMC neurons since re-expression of LepRb selectively in POMC neurons, under which condition no developmental compensation will occur, produces little effects on body weight while largely restores euglycemia in *db/db* mice [19, 20]. However, it could be the case for AgRP neurons since lesion of these neurons in neonatal stages produces little effects while that in adulthood leads to starvation [21]. Thus, further studies with inducible deletion of LepRb in AgRP neurons in adulthood are required to assess direct action of AgRP neurons in mediating leptin action on body weight. The other possibility is that there exist other unidentified groups of neurons in the Arc, which play more important roles in mediating leptin action. The mild role of LepRb in POMC and AgRP neurons is simply due to the fact that these neurons only account for a fraction of total LepRb-expressing neurons in the Arc. Consistent with a minor role of POMC neurons in mediating leptin action, only a fraction of POMC neurons express LepRb [22]. The existence of unidentified LepRb-expressing neurons in the Arc is supported by recent data that leptin activation of POMC neurons is indirectly mediated by reducing GABA release to these neurons [23•]. Importantly, AgRP neurons are not a significant part of the GABAergic neuron since disruption of GABA release from AgRP neurons produces little effects on leptin activation on POMC neurons. Given the fact that Arc neurons are mainly GABAergic neurons and LepRb is mostly abundantly expressed in the Arc, the GABAergic neurons that directly respond to leptin are most likely

located in the Arc. Future efforts should be directed to understand the role of non-POMC, non-AgRP neurons in the Arc in mediating leptin action.

Earlier lesion studies suggest the VMH as a satiety center in the brain. Consistently, defects in VMH development by deletion of steroidogenic factor 1 (SF1), a transcription factor required for VMH development, lead to massive obesity and hyperphagia. Further, specific deletion of SF1 in adult VMH also causes obesity [24], suggesting a role for SF1 in maintaining the role of VMH neurons in obesity regulation. Consistent with prominent expression of LepRb in the VMH, the VMH shows prominent p-STAT3 expression in response to leptin, suggesting an important role for VMH neurons in mediating leptin action. Indeed, specific deletion of LepRb in the VMH causes obesity associated with hyperphagia [18]. However, compared to those of VMH/SF1 lesion on obesity, the effect of LepRb deletion in the VMH is mild. These results suggest that the VMH only mediates part of leptin action on body weight and that the VMH mediates the action of other important factors for body weight homeostasis.

Both DMH and lateral hypothalamus express abundant LepRb and shows robust p-STAT3 expression in response to leptin action [13], suggesting an important function for LepRb-expressing neurons in these regions in mediating leptin action. Despite this, the specific role of LepRb in these regions remains elusive due to lack of means to genetically target these regions, i.e., mouse strains with specific Cre expression in these regions. Nonetheless, accumulating evidence suggests a role for these neurons in mediating body weight homeostasis. DMH LepRb neurons receive prominent inputs from preoptic neurons and send strong projections to the brown adipose tissue (BAT) [25], indicating a role for these neurons in energy expenditure. Consistently, a role for these neurons in mediating leptin action on thermogenesis in diet-induced obesity mice has been demonstrated [26•]. These data suggest a functional role for these DMH LepRb-expressing neurons in energy expenditure regulation. Interestingly, in the lateral hypothalamus, LepRb-expressing neurons express neurotensin and are largely GABAergic; these neurons are neither orexin nor MCH positive, but instead lie upstream of orexin and MCH neurons, providing GABAergic innervations [27•]. Importantly, the LepRb-expressing neurons in the lateral hypothalamus also project to ventral tegmental area (VTA) dopaminergic neurons and this projection is important for feeding regulation by modulating dopamine release.

Recent studies also demonstrated an important role for those LepRb-expressing neurons that are located outside the hypothalamus. LepRb is expressed in a small subset of VTA neurons. Although it remains unclear how leptin directly modulates dopamine action, convergent evidence suggests that dopamine may mediate leptin action on locomotion and

hedonic aspects of food consumption [28]. LepRb is also expressed a subset of hindbrain neurons, especially in the nucleus of solitary tract (NTS) area. Peripheral co-administration of leptin and cholecystokinin (CCK), a gut derived hormone, causes a synergistic effect in reducing feeding [29], suggesting the existence of a common subset of neurons that express both LepRb and CCK receptors, which are likely located in the hindbrain/NTS area. Consistently, specific deletion of LepRb in the NTS area leads to obesity, demonstrating a role for NTS LepRb-expressing neurons in body weight regulation [30]. Surprisingly, NTS deletion of LepRb causes no alternations in food intake.

Neurotransmitters Mediating Leptin Action

One key step in understanding leptin neural pathways is to reveal the neurotransmitters that mediate the function of important LepRb-expressing neurons. In the Arc, it is well established that α -MSH released from POMC, and AgRP and NPY released from AgRP neurons mediate leptin action. Compelling pharmacological data demonstrate that AgRP and NPY, when administered in the brain, produce powerful orexigenic effects, while α -MSH and its mimetic, MTII, produce anorexigenic effects [31]. Electrophysiological data demonstrate that leptin activates POMC neurons while inhibiting AgRP neurons [32]. In further corroboration with this, fasting induces robust up-regulation of AgRP and NPY, and down-regulation of POMC, which coincides with decreased levels of leptin [33], suggesting that the underlying mechanism for fasting-induced hyperphagia is inhibition of POMC neurons and “dis-inhibition” of AgRP neurons as a result of reduced leptin levels. Genetically, genetic knockout of POMC or over-expression of AgRP (agouti mice) leads to severe obesity [34], demonstrating a powerful role for the melanocortin system in body weight regulation. In addition, β -endorphin and adrenocorticotropin (ACTH), both of which are encoded by the same POMC gene, cocaine- and amphetamine-regulated transcript peptide (CART), galanin-like peptide (GALP) and kisspeptin appear also to be released from LepRb neurons in the Arc; however, their function in mediating leptin function remains to be established. In the VMH, expression levels of brain-derived neurotrophic factor (BDNF) responds to nutritional status and deletion of VMH BDNF leads to obesity and hyperphagia [35, 36], suggesting that BDNF mediates the action of VMH neurons in the regulation of energy balance. In addition, recent results indicate that pituitary adenylate cyclase activating polypeptide (PACAP) is abundantly expressed in the VMH and its expression levels respond to nutritional status, suggesting a potential role for PACAP in body weight regulation [37]. NPY expression in the DMH appears to regulate body weight [38]; however, it remains to

ascertain the extent to which DMH NPY mediates leptin action. No neuropeptide has yet been implicated in directly mediating leptin action in the lateral hypothalamus as well as other brain regions.

Given the importance of fast-acting neurotransmission in general, fast-acting neurotransmitters released from LepRb neurons are probably the major mediators for leptin action. Notably, key hypothalamic regions with abundant LepRb expression are mainly glutamatergic or GABAergic [39]. The Arc expresses abundant vesicular GABA transporter (VGAT), but little vesicular glutamate transporter 2 (VGLUT2), suggesting the majority of Arc neurons are largely GABAergic. The VMH and PMv express abundant VGLUT2, but little VGAT, suggesting that neurons in these regions are largely glutamatergic. The DMH and POA express both VGLUT2 and VGAT, suggesting that neurons in these brain sites are composed of both glutamatergic and GABAergic neurons (see the diagram in Fig. 1).

Based on the animal model with LepRb deletion in GABAergic neurons, a recent study revealed that LepRb in GABAergic neurons mediate a major part of leptin role on body weight regulation [23••], suggesting a major role for GABA release in mediating leptin action. In contrast, specific disruption of GABA release from LepRb neurons leads to mild obesity associated with hyperphagia and reduced energy expenditure [40••]. These results may lead to speculations that other neurotransmitters released from LepRb-expressing neurons play an important role or that disruption of GABA release from different subsets of LepRb-expression causes opposite effects, i.e., body weight-reducing effect from leptin-inhibited neurons and body weight-increasing effect from leptin-excited neurons. Consistent with this idea, disruption of GABA release from AgRP neurons, well established leptin-inhibited neurons, leads to reduced body weight associated with increased energy expenditure [41]. Further studies with disruption of GABA release from each distinct subset of LepR-expressing neurons will provide more insights on GABAergic action in mediating leptin function.

Surprisingly, despite its strong expression in the VMH, deletion of VGLUT2 in the VMH leads to normal body weight on chow and only slightly increased body weight on high fat diet, suggesting a role for other neurotransmitters in mediating body weight action of VMH neurons [42]. Clearly, further studies are required to examine the role of glutamate release from the PVH and PMv, and glutamate and GABA release from the DMH and POA.

Leptin Neural Pathway for Reproduction

It is well established that nutrition and reproduction are tightly coordinated. On one hand, a minimum amount of

stored energy is required for normal pubertal development and to maintain the tone of the reproductive system [43, 44]. When survival is threatened by unavailability of food or increased energy demands, males and females of most species divert energy away from reproduction-related processes. These include pubertal development, the production of reproductive hormones and gametes, and the maintenance of pregnancy and lactation. On the other hand, excess energy also has a negative impact on the reproductive physiology. For example, elevated adiposity in women aggravates polycystic ovarian syndrome and ovulatory dysfunctions and may induce hypothalamic hypogonadism [45, 46]. Moreover, the increasing rates of childhood obesity have been associated with the advance in the timing of pubertal maturation and its deleterious consequences [47–50]. Collectively, these findings predict that factors must exist to regulate energy homeostasis and reproductive functions in a coordinated fashion.

The adipocyte-derived hormone leptin is certainly one suitable signal that provides the fundamental link between energy balance and reproduction. For example, obesity seen in mice lacking leptin (*ob/ob*) or LepRb (*db/db*) is accompanied by infertility [51–53]. These mice also exhibit low luteinizing hormone (LH) levels and incomplete development of reproductive organs and do not undergo puberty. Leptin administration to *ob/ob* mice can completely rescue all these phenotypes [54–56]. Further, humans with leptin deficiency develop similar reproductive phenotypes as seen in *ob/ob* mice [6]. In these patients, leptin replacement induces an increase in the levels of gonadotropins and sex steroids as well as enlargement of the gonads and normal pubertal development [57, 58]. Leptin also overrides the fasting-induced suppression of LH secretion and fertility [59–62]. In anorectic females and in athletes with extreme decreases in body adiposity, leptin can increase levels of LH [63] and restore the menstrual cycle [64]. Clearly, leptin serves as a key circulating molecule that mirrors the internal energy storage and therefore produces a permissive signal for normal reproductive functions. In conditions that leptin signals are impaired due to lack of energy store or genetic mutations, reproductive functions are compromised as a defending mechanism to reserve energy and increase survival.

Re-expression of LepRb in the brain of LepRb-null mice restores fertility completely in males and partially in females [65, 66]. These indicate that leptin acts primarily via LepRb in the brain to regulate reproduction. An interesting question is whether leptin-mediated regulations on energy balance and on reproduction are mediated by the same or overlapping neural circuits. While many LepRb-expressing neural populations have been identified as critical regulators of body weight (as discussed above), our efforts to identify LepRb sites important for reproduction have just begun.

Among many sites that deserve investigation, the most studied LepRb site in the context of reproduction is perhaps the PMv. The PMv expresses abundant leptin receptors and projects directly to gonadotropin releasing hormone (GnRH) neurons [67, 68]. Elias and coworkers reported that adult female rats with bilateral lesions of the PMv are unresponsive to leptin's effect to induce LH secretion [69]. They further demonstrated that while exogenous leptin can rescue development of puberty in *ob/ob* mice with intact PMv, bilateral lesions of the region blunted the effects of leptin [70•]. Importantly, unilateral re-expression of endogenous LepRb in PMv neurons was sufficient to induce puberty and partially improve fertility in female *db/db* mice [70•]. The re-expression of LepRb in the PMv also normalized the increased hypothalamic GnRH content characteristic of leptin-signaling deficiency [70•]. These data suggest that LepRb expressed in the PMv is *sufficient* to mediate leptin's action at the onset of puberty.

The role of LepRb in the PMv was further supported by a recent report by Myers and coworkers [71•]. They first identified that about 20 % of LepRb-positive neurons express nitric oxide synthase 1 (NOS1), an enzyme responsible for production of nitric oxide in neurons. Interestingly, these LepRb-positive NOS1 neurons are predominantly in the PMv, with a few of them located in the DMH and Arc. Notably, the LepRb-positive NOS1 neurons in the Arc are distinct from POMC and AgRP neurons. Importantly, female mice lacking LepRb selectively in NOS1 neurons showed delayed puberty, although their fertility did not appear to be affected. Nevertheless, Myers observations indicate that LepRb in the PMv (as well as in the DMH and Arc) is *required* for the normal development of puberty. Together with Elias's findings, these results identified the PMv as the key site where leptin signals are both required and sufficient to regulate pubertal development. Importantly, mice lacking LepRb in NOS1 neurons also developed severe obesity [71•]. Thus, the PMv may serve a critical node where the metabolic and reproductive signals are coordinated.

It is noted, however, that LepRb in the PMv is neither sufficient [70•] nor required [71•] to mediate leptin's effects on fertility. This suggests that leptin acts on anatomically dissociated LepRb populations to regulate pubertal development and fertility. POMC neurons may be the direct target of leptin and insulin actions important for fertility. Hill and coworkers reported that female mice lacking both leptin and insulin receptors in POMC neurons exhibit lengthened reproductive cycles, follicular arrest, hyperandrogenemia, and are sub-fertile [72•]. While hypothalamic GnRH gene expression was normal, LH levels were significantly increased in females. Histological examination of their ovaries showed that double knockout females exhibited more degenerating follicles. Serum testosterone

levels were significantly elevated in females, accompanied by a significant elevation in the expression of ovarian 3 β -HSD I gene, which produces androstenedione. Collectively, these results suggest that the absence of leptin and insulin signaling in POMC neurons may reduce the inhibitory tone on GnRH neurons and cause basal LH levels to increase, disrupting reproductive function. Interestingly, such reproductive phenotypes were not observed in mice lacking either receptor alone in POMC neurons, suggesting that signals initiated by leptin and insulin in POMC neurons are functionally redundant in the context of fertility.

Another possible site that mediates leptin actions on reproduction is Kiss1 neurons in the Arc. About 15 % Kiss1 neurons in the Arc respond to leptin with activation of the STAT3 pathway [73]. Leptin-deficient *ob/ob* male mice show decreased expression of *Kiss1* in the Arc, which is increased by leptin treatment [74]. Hypothalamic *Kiss1* mRNA levels are decreased in male rats made diabetic by administration of streptozotocin [75]. Intracerebroventricular administration of leptin normalizes *Kiss1* gene expression and the levels of LH and androgens. However, genetic deletion of LepRb selectively from hypothalamic Kiss1 neurons in mice had no effect on puberty or fertility [70•]. These results may indicate that direct leptin signaling in Kiss1 neurons is not required for these processes. Alternatively, the lack of reproductive phenotypes in mice with embryonic deletion of LepRb from Kiss1 neurons may be due to the existence of compensatory pathways. Indeed, recent studies showed that embryonic ablation of neurons expressing Kiss1 or its receptor (GPR54) did not affect puberty and fertility, while acute ablation of Kiss1 neurons from adult mice inhibited fertility [76]. Thus, the requirement of leptin signaling on Kiss1 neurons for normal pubertal development has not been fully excluded and deserves further investigation.

Conclusions

In summary, exciting progress has been made in our understanding of the neural pathway that mediates leptin action. Given the demonstrated roles of leptin receptor-expressing neurons in body weight in the Arc, VHM, LH, VTA and the hindbrain, it appears that leptin action on body weight is mediated by a widely distributed neural network. It remains unknown whether LepRb-expressing neurons in the DMH, POA and PMv also mediate leptin action on body weight. However, based on recent observations that LepRb in both GABAergic neurons and NOS1 expressing neurons mediates predominantly leptin action on body weight [23•, 71•], a small subset of GABAergic LepRb neurons located in the Arc may play a major part in mediating leptin action. Further studies are required to identify this subset of neurons. For

fertility regulation, it is unknown to what extent fertility is directly regulated by leptin action and indirectly associated by the obesity caused by defects in leptin action. Previous studies suggest that mice with impaired LepRb $\rightarrow$ STAT3 signaling exhibit the same degree of obesity as in *ob/ob* mice, but maintain fertility, suggesting a direct role for leptin action in fertility [5]. Interestingly, a recent study found that while AgRP neurons are not necessary for fertility, acute lesion of these neurons can rescue the fertility in leptin-deficient *ob/ob* mice [77•]. Since the rescuing effect in fertility is accompanied by rescued body weight, this result argues that infertility in *ob/ob* mice may also be a secondary consequence of obesity. Further studies are warranted to dissect leptin-direct and –indirect pathways for fertility regulation.

It is well established that leptin action in the hypothalamus is critical for body weight homeostasis. However, the vast majority of obesity is associated with high leptin levels, indicative of leptin resistance. Thus, strategies aiming at reversing leptin resistance, activating post-leptin resistance signal step and bypassing leptin resistance represent promising therapeutics for obesity treatment. A large body of evidence suggests multiple molecules and intracellular leptin signaling pathways as leptin resistance sites [78]. Neurotransmitter release and its action on postsynaptic neurons represent major post-leptin resistance steps. With the advent of new technologies, more neurotransmitters and new function of existing neurotransmitters will be identified, which will lead to delineation of brain mechanisms governing energy balance and provide novel targets for specific and effective drugs to reverse and prevent the current devastating obesity epidemic and its associated infertility.

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